

For PPI-Induced ERK Signaling, see Figure 1B, Panel B, and Figure 2C.

1. Introduction

ERK1/2, also known as extracellular signal-regulated kinase 1/2, is a member of the mitogen-activated protein kinase (MAPK) family. It is composed of two closely related proteins, ERK1 (42 kDa) and ERK2 (44 kDa). ERK1/2 is activated by various extracellular stimuli, such as growth factors, cytokines, and stress signals, through receptor-mediated or non-receptor-mediated pathways. The activation of ERK1/2 leads to a variety of biological responses, including cell proliferation, differentiation, and apoptosis. In addition, ERK1/2 has been implicated in the regulation of gene expression, protein phosphorylation, and lipid metabolism.

2. Activation Mechanisms

The activation of ERK1/2 is primarily mediated by the Ras/Raf/MEK/ERK signaling pathway. This pathway consists of four main components: Ras, Raf, MEK, and ERK. Upon activation, Ras-GTP binds to Raf, which triggers the phosphorylation of Raf at multiple sites, leading to its activation. Activated Raf then activates MEK, which in turn phosphorylates ERK1/2 at its T-loop domain. The activated ERK1/2 dimer then translocates to the nucleus and regulates gene expression. Other activation mechanisms include the JNK/SAPK pathway, PI3K/Akt pathway, and various G-protein coupled receptor (GPCR)-mediated pathways.

3. Experimental Protocols

3.1. Cell Culture and Treatment

Cells are seeded onto a 100 mm dish and allowed to grow until they reach 80% confluence. Cells are then treated with various concentrations of PPIs (e.g., omeprazole, pantoprazole, esomeprazole, rabeprazole, and lansoprazole) for different time intervals (e.g., 1, 3, 6, and 12 hours). Control cells are treated with vehicle only.

3.2. Western Blot Analysis

Cells are lysed in RIPA buffer containing protease inhibitors. Lysates are separated by SDS-PAGE and transferred to PVDF membranes. Membranes are probed with antibodies specific for ERK1/2, p-ERK1/2, Raf, MEK, and phospho-Raf. Immunoblotting is performed using a chemiluminescence detection system.

3.3. Immunofluorescence

Cells are fixed in 4% paraformaldehyde and permeabilized with Triton X-100. Cells are then stained with antibodies against ERK1/2 and p-ERK1/2. Nuclei are counterstained with DAPI. Fluorescence microscopy is used to visualize the distribution of ERK1/2 and p-ERK1/2.

3.4. RT-PCR

mRNA is isolated from cells using Trizol reagent. cDNA is synthesized using reverse transcriptase. Real-time PCR is performed using primers specific for ERK1/2 and GAPDH. The relative mRNA levels are calculated using the $\Delta\Delta C_t$ method.

3.5. Luciferase Assay

Cells are transfected with a luciferase reporter construct containing the promoter region of the ERK1/2 gene. Cells are then treated with PPIs and luciferase activity is measured using a luminometer.

4. Data Analysis

All data are expressed as mean \pm SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ is considered statistically significant.

5. Conclusion

This protocol provides a detailed guide for the study of PPI-induced ERK signaling. By following these steps, researchers can effectively investigate the molecular mechanisms underlying PPI-induced signaling and identify potential therapeutic targets.



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